Radboud Repository

Radboud University Nijmegen

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/22132

Please be advised that this information was generated on 2018-07-07 and may be subject to change.

Growth Factors in Experimental Osteoarthritis: Transforming Growth Factor ß Pathogenic?

WIM B. van den BERG

W.B. van den Berg

ABSTRACT. Osteoarthritis (OA) is characterized by focal cartilage destruction and osteophyte formation. Triple injections of transforming growth factor ß (TGFß) in normal murine knee joints revealed that this growth factor can elicit osteophytes at sites characteristic for OA. Moreover, enhanced cartilage proteoglycan synthesis is induced and cartilage proteoglycan content is increased. Exuberant repair reactions may be pathogenic and indeed focal cartilage proteoglycan loss and disorganized chondrocyte spacing is noted in the tibial plateau after 1 month. Although TGFß is immunosuppressive and can counteract interleukin 1 induced cartilage damage, suggesting potential therapeutic application in joint inflammation, it should probably be viewed as a pathogenic factor in OA. (*J Rheumatol 1995;*(**suppl 43)22:143–5**)

Key Indexing Terms:
TGFß PROTEOGLYCAN SYNTHESIS OSTEOPHYTES REPAIR

Osteoarthritis (OA) is characterized by focal cartilage destruction and changes in the underlying bone. Apart from sclerosis, formation of new cartilage and bone occurs at the edges of articular structures and at insertion sites of ligaments and tendons. These so-called osteophytes may help to protect the damaged joint against ongoing biomechanical destruction. The general appearance of processes in OA cartilage underlines a failure in attempt at repair. Although the pathogenesis is far from understood, it is quite likely that we deal with common endpoints of multiple causes. In addition to genetic disturbances in cartilage enzymes and molecular structure of matrix macromolecules, factors like joint instability and blunt trauma to cartilage have been identified to contribute to development of OA. Animal models are helpful to elucidate mechanisms and mediators, or to study the potential application of growth factors in prevention of ongoing damage and improvement of adequate repair. Cartilage homeostasis is controlled by endogenous cytokines and growth factors. It is known that insulin like growth factor (IGF-1) is a major anabolic regulator enhancing chondrocyte proteoglycan synthesis and inhibiting normal proteoglycan breakdown[1,2]. In addition, transforming growth factor ß (TGFß) is an intriguing modulatory factor. It is known from *in vitro* studies that it has no or an inhibitory effect on proteoglycan synthesis in freshly isolated chondrocytes, whereas it has a clear stimulatory effect after plain preculture or prolonged exposure to TGFß, and it is tempting to suggest that this change is related to a shift in receptor expression[3,4]. Apart from general enhancement of aggrecan synthesis, it predominantly enhances the synthesis of small proteoglycans such as decorin. In addition, TGFß counteracts interleukin 1 (IL-1) effects on cartilage through downregulation of IL-1 receptors, upregulation of proteoglycan synthesis, and release of enzyme inhibitors and IL-1 receptor antagonist. We have examined the effect of repeated intraarticular TGFß injections in the murine knee joint, either alone or in combination with IL-1. A single injection of TGFß has no major effect, but triple injections given on alternate days markedly enhanced cartilage proteoglycan synthesis, which remained high for several days. This was found both in young and in aged mice (Figure 1). When given in combination with IL-1, it clearly counteracted the IL-1 mediated proteoglycan synthesis inhibition[5,6]. This effect is also illustrated in the measurement of proteoglycan content. At Day 4 after the last injection the IL-1 exposed joint showed marked cartilage proteoglycan depletion, whereas the IL-1/TGFß combination appeared almost normal (Figure 1). Intriguingly, TGFß alone caused a significant rise in proteoglycan levels above the normal content.

Another striking observation was the clear development of osteophytes after repeated TGFß injections. Within 4 days after the last injection, outgrowth of chondroid tissue is noted at the margins of the cartilage (Figure 2) and at insertion sites of ligaments. In fact, these sites correlate with localization of osteophytes in murine OA models[6,7]. Further outgrowth to bony structures, containing bone marrow, is noted after a few weeks. Of interest, IL-1 antagonizes the stimulating effect of TGFß on chondrocyte proteoglycan synthesis in preexisting cartilage, but does not seem to interfere with osteophyte formation. Osteophytes were as marked in the IL-1/TGFß combination group and were absent in the IL-1 mice.

We further examined the potential difference between TGFß1 and TGFß2, and the capacity of IGF-1 to mimic the *in vivo* events mentioned above. Effects of TGFß1 and

From the Department of Rheumatology, University Hospital Nijmegen, Nijmegen, The Netherlands.

W.B. van den Berg, PhD, Professor of Experimental Rheumatology, University Hospital Nijmegen.

Address reprint requests to Prof. W.B. van den Berg, Dept. of Rheumatology, University Hospital Nijmegen, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, The Netherlands.

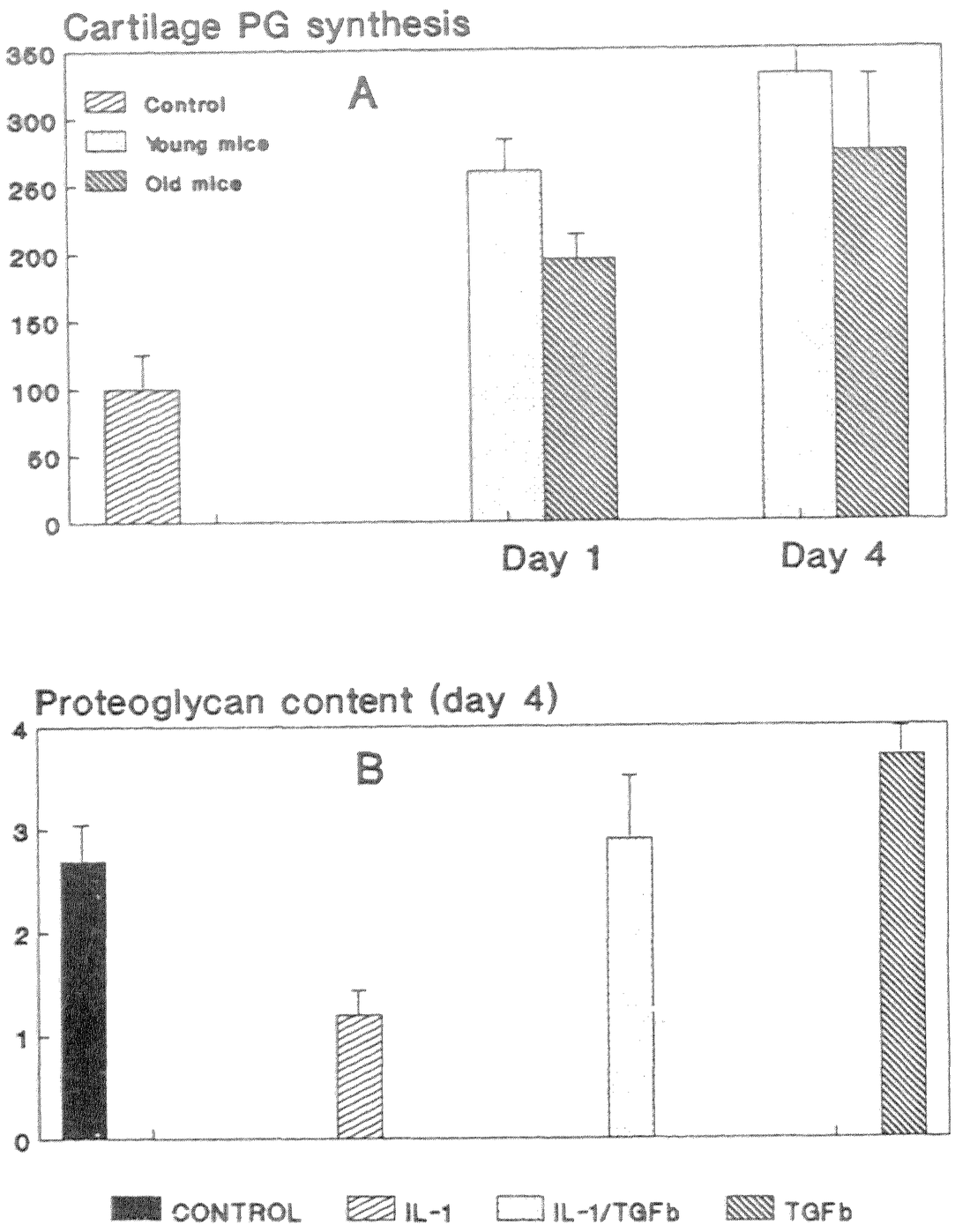

Fig. 1. A. Proteoglycan synthesis in patellar cartilage at various days after the last of triplicate intraarticular injections of TGFß (100 ng), given alternate days. This is measured *ex vivo* using radiolabelled sulfate incorporation in a 2 h culture period. Mice were either 3 months (young) or 18 months (old). B. Proteoglycan content of the patellar cartilage of young mice at Day 4 after the last of triple injections with IL-1 (1 ng), TGFß (100 ng), or the combination (protocol as above). This was measured on saffranin-O stained joint sections with an automated image analysis system[6].

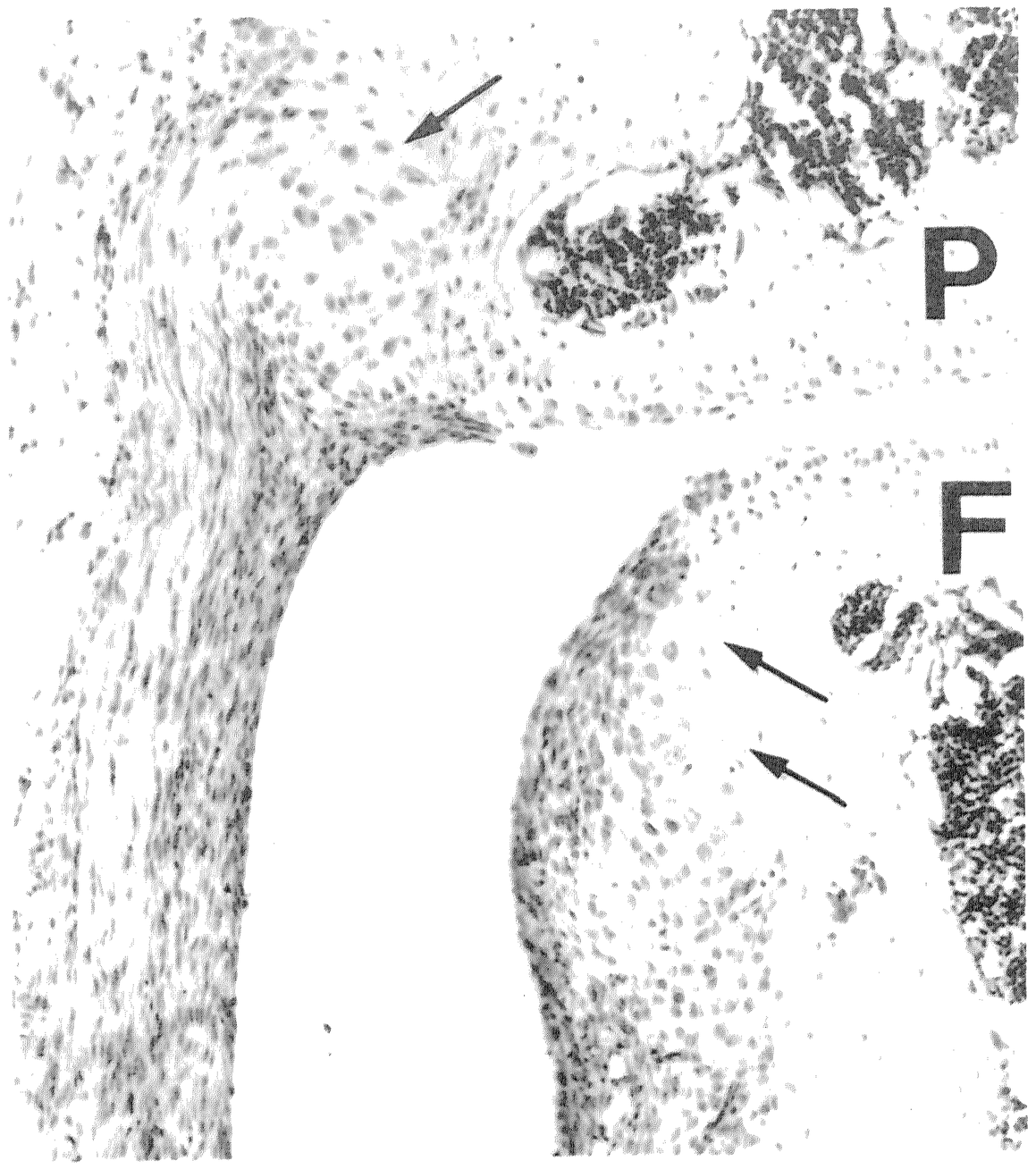

Fig. 2. Histology of the knee joint at Day 4 after the last of triple intraarticular injections with TGFß. Details as in Figure 1. Note the chondroid outgrowth at the margins of the patella and femur. Arrows indicate the original lining of the joint.

TGFß2 on both chondrocyte proteoglycan synthesis and induction of osteophytes were indistinguishable. IGF-1 only induced a small stimulation of chondrocyte proteoglycan synthesis, and counteraction of IL-1 effects was minor compared with TGFß. IGF-1 did not induce osteophytes. Although IGF-1 is a major anabolic factor *in vitro,* the endogenous levels are already high in the synovial fluid, which makes it difficult apparently to generate a substantial increase by local administration. In contrast, most of the endogenous TGFß will be in a latent form, expecting that local injection with active TGFß makes a substantial difference.

Observations in experimental OA models point to initial cartilage hypertrophy and enhanced chondrocyte proteoglycan synthesis[8,9]. At later stages loss of cartilage matrix, enhanced expression of proteolytic enzymes, and imbalance with natural inhibitors is noted, but even then the remaining chondrocytes quite often show enhanced proteoglycan synthesis, apparently attempting to replenish the depleted matrix. This is hardly compatible with a prominent role of IL-1 and suggests at least the dominant generation and/or activation of stimulatory factors. Given the profile of TGFß effects mentioned above, this growth factor seems a likely candidate. It is therefore tempting to speculate that TGFß is a pivotal pathogenic mediator in OA. In fact, careful examination of histological knee joint sections revealed first signs of OA-like lesions in the tibial plateau at 4 to 8 weeks after triple TGFß injections. In these mice, aged 3–4 months, typical loss of proteoglycan staining close to the tide mark and disorganization of chondrocyte spacing is consistently found in the TGFß injected joints, whereas the contralateral, saline injected control joints of these mice remain without symptoms. Similar changes can be found in the tibial cartilage of untreated C57Bl mice at later age. We have yet to identify whether TGFß only enhances the expression in strains prone to develop spontaneous OA, such as the C57Bl mice, or whether these cartilage lesions can also be induced in nonsusceptible strains.

Considering joint instability as a prominent cause of OA, the following principles might be involved in full development of lesions (Figure 3). Instability may implicate substantial overloading, causing direct cartilage or bone injury and, in the presence of inadequate repair, this may lead to OA. The latter pathway is also involved in OA after blunt cartilage trauma. On the other hand, in instability models, using ligament sectioning or collagenase induced ligament damage, the evidence for plain injury to the cartilage is lack-

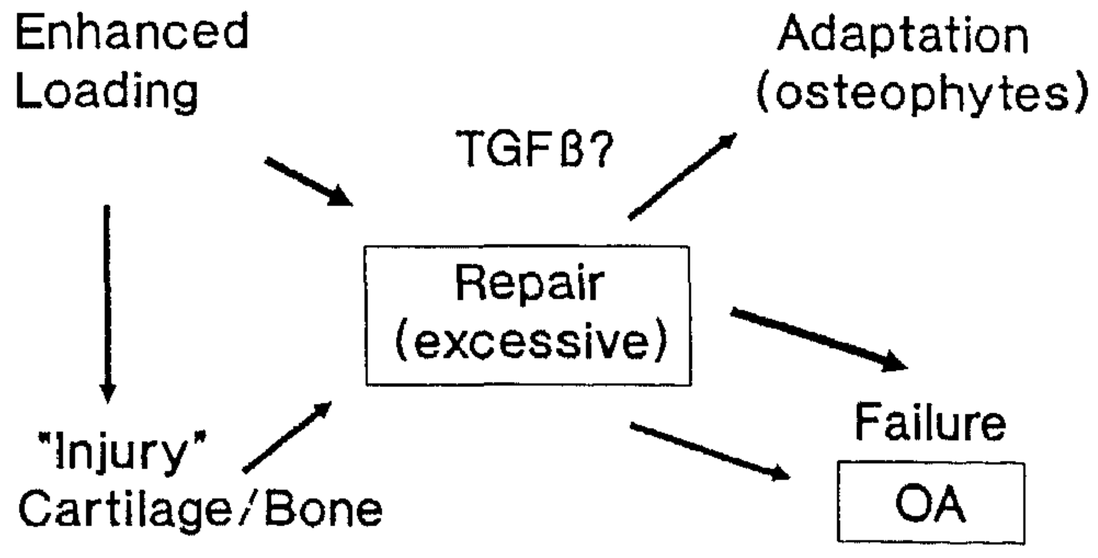

Fig. 3. Principles involved in instability induced OA.

ing. An alternative pathway may therefore be that overloading or the repair reaction itself leads to excessive TGFß generation/activation. This leads to excessive cartilage proteoglycan content, which either alone or in the presence of overloading, may cause ultimate, focal cartilage damage. Given that TGFß seems a pivotal mediator in osteophyte formation, indirect evidence for its activation in the OA joint is already provided. Future studies with TGFß specific inhibitors or receptor blockers are needed to fully prove its precise involvement.

Therapy with growth factors remains a challenging option to improve the outcome of OA lesions. Studies with IGF-1 in combination with oversulfated glycosaminoglycan (GAG) have already proven efficacy in the rabbit meniscectomy model of OA[10]. However, one should be careful with TGFß. It is tempting to speculate that limited TGFß therapy may be of benefit in RA lesions, counteracting IL-1 and suppressing T cell reactions, but too much TGFß might well have unwanted side effects on the cartilage, in particular under OA conditions. Another complicating factor is formed by a shift in receptor expression and apparent nonresponsiveness to further stimulation with growth factors, as noted in arthritis and OA cartilage[11,12]. Better understanding of subtypes of receptors involved in various cell activation processes may perhaps yield a more selective approach. In addition, the recent findings with the cloned bone morphogenetic proteins, which belong to the TGFß superfamily and show similar but also additional activities above TGFß and IGF-1 effects, may promise alternative therapies.

REFERENCES

1. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC: Stimulation of proteoglycan biosynthesis by serum and IGF-1 in cultured bovine articular cartilage. *Biochem J 1986;*240:423–30.
2. Schalkwijk J, Joosten LAB, van den Berg WB, van Wijk JJ, van de Putte LBA: IGF stimulation of chondrocyte proteoglycan synthesis by human synovial fluid. *Arthritis Rheum 1989;*32:66–71.
3. van der Kraan PM, Vitters EL, van den Berg WB: Differential effect of TGFß on freshly isolated and cultured articular chondrocytes. *J Rheumatol 1992;*19:140–5.
4. Glansbeek HL, van der Kraan PM, Vitters EL, van den Berg WB: Correlation of the size of type II TGF-ß receptor with TGF-ß responses of isolated bovine articular chondrocytes. *Ann Rheum Dis 1993;*52:812–6.
5. van Beuningen HM, van der Kraan PM, Arntz OJ, van den Berg WB: Protection against IL-1 induced articular cartilage damage by TGFß *in vivo:* Age related differences. *Ann Rheum Dis 1994;*53:593–60.
6. van Beuningen HM, van der Kraan PM, Arntz OJ, van den Berg WB: TGFß1 stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint. *Lab Invest 1994;*71:279–90.
7. van den Berg WB, van Osch GJVM, van der Kraan PM, van Beuningen HM: Cartilage destruction and osteophytes in instability-induced murine osteoarthritis: Role of TGFß in osteophyte formation. *Agents Actions 1993;*40:215–9.
8. Brandt K: Transection of the anterior cruciate ligament in the dog. A model of osteoarthritis. *Semin Arthritis Rheum 1991;*21:22–32.
9. van der Kraan PM, Vitters EL, van Beuningen HM, van den Berg WB: Proteoglycan synthesis and osteophyte formation in ''metabolically'' and ''mechanically'' induced murine degenerative joint disease. *Int J Exp Pathol 1992;*73:335–50.
10. Howell DS, Altman RD: Cartilage repair and conservation in osteoarthritis. *Rheum Dis Clin North Am 1993;*19:713–24.
11. Doré S, Pelletier JP, DiBattista JA, Tardif G, Brazeau P, Martel-Pelletier J: Human osteoarthritic chondrocytes possess an increased number of IGF-1 binding sites but are unresponsive to its stimulation. *Arthritis Rheum 1994;*37:253–63.
12. Verschure PJ, Joosten LAB, van der Kraan PM, van den Berg WB: Responsiveness of articular cartilage from normal and inflamed mouse knee joints to various growth factors. *Ann Rheum Dis 1994;*53:455–60.